

Response of the wasp *Cephalonomia tarsalis* to *Beauveria bassiana* as free spores or infection in its host, the sawtoothed grain beetle, *Oryzaephilus surinamensis*.

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Cephalonomia tarsalis (Bethyilidae) is a predator and ectoparasitoid of larval sawtoothed grain beetles (STGB). The beetle larvae are also very susceptible to *Beauveria bassiana*, a registered mycoinsecticide with excellent prospects for use against grain storage and processing pests. This work addresses the compatibility of the two biological control agents.



Cephalonomia tarsalis manipulating a sawtoothed grain beetle larva. Adult wasps feed on as well as parasitize the beetle lar-



Larval sawtoothed grain beetle infected with the fungus *Beauveria bassiana* (reddish pigment) and an egg of the parasitic wasp *Cephalonomia tarsalis*. The wasp will die within 24 hours.

Methods

Wasps: Female wasps 4-7 days post-emergence and held for at least 24 hours in the presence of males were used in all experiments. Chill anesthesia was used for handling.

Fungus: Commercially produced, unformulated conidia of *Beauveria bassiana* isolate GHA were obtained from Mycotech Corp., Butte MT. The conidia powder contained 6.3×10^{10} spores/g, and a germination rate above 90% in all tests.

Beetles and fungus treatments: Beetles from the GMPRC colony were reared to the last instar on rolled oats with brewer's yeast at 30°C. For fungus exposures, they were placed in a beaker with 1 g of ground rolled oats and 5 mg of *B. bassiana* spores and incubated at 100% RH chamber at 26°C for 24 hours.

No choice oviposition: Female wasps were placed individually in 12.5 cm ventilated culture flasks with 3 beetle larvae. The presence and condition of wasp eggs and larvae and the condition of the hosts were monitored daily. Fungus treatments were 0, 1, and 2 days of exposure. There were 30 replicates in each of 3 repetitions of the experiment.

Choice test for oviposition: Beetle larvae were either untreated or exposed to fungus for 1 or 2 days but not showing red pigment from infection. Groups of 3 beetles were placed in 25 100 ml beakers per treatment with 5 g of hard red winter wheat. The beakers were placed randomly in a large plastic bin and 68-78 wasps were released in the center. Oviposition was assessed after 24 hr of incubation and the treated larvae were classified has red or not. Dried out, dead larvae, clearly unsuitable for oviposition were not included in the results. The experiment was run three times.

Choice test for entry into fungus-treated grain: Wasps were placed in the center of 150 mm petri dishes among three 60 mm plastic petri dishes with 0, 100, or 500 ppm *B. bassiana* conidia in 20 g of wheat. Each small dish also contained 4 large STGB larvae. The wasps were watched for 10 min and timed for residence in each treatment. A total of 108 wasps were observed entering the treated wheat.

Adult wasp mortality: Groups of 20 female wasps were placed on 100 g of wheat kernels with 0, 100, or 500 ppm *B. bassiana* conidia for 3 hours. They were then transferred to 100 g of clean wheat with 40 fourth instar STGB, incubated for 8 days at 26°C and 75% RH, and scored for survival. The test was repeated three times.

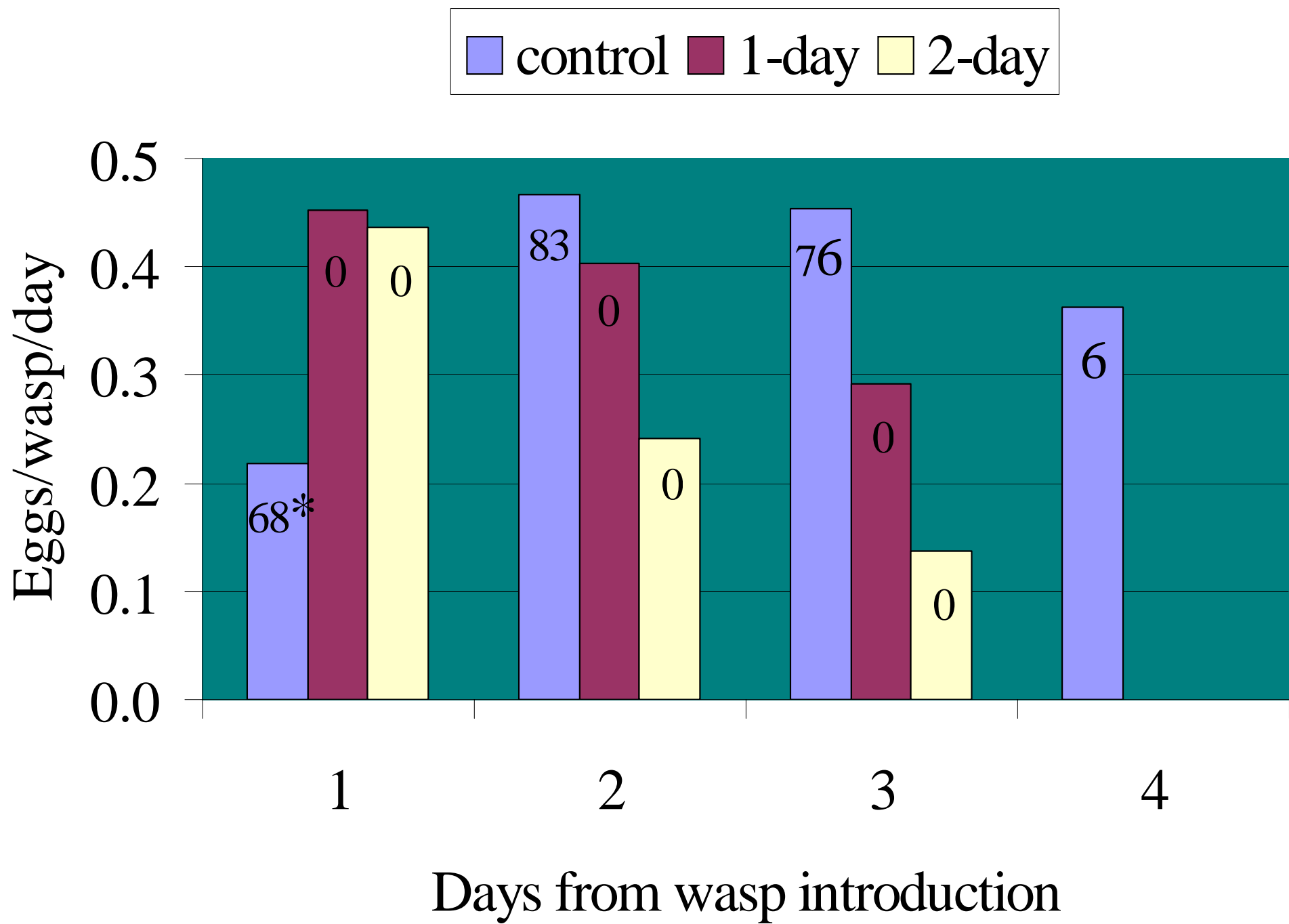


Figure 1. No choice oviposition of *Cephalonomia tarsalis* on sawtoothed grain beetles that had 1 or 2 days prior treatment with *Beauveria bassiana*. *Percent of progeny that survived to day 7.

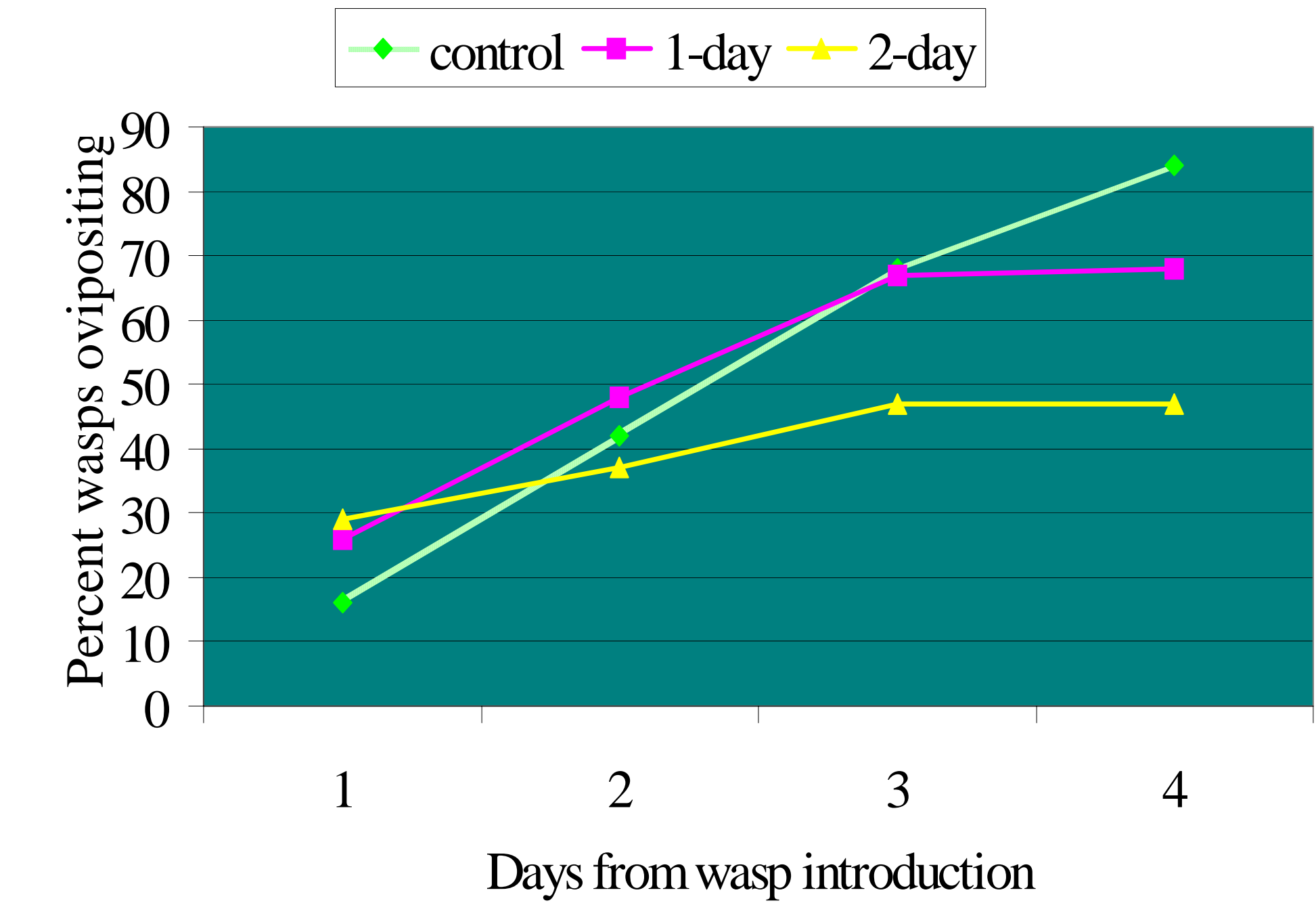


Figure 2. Cumulative percent *Cephalonomia tarsalis* oviposition on sawtoothed grain beetles treated with *Beauveria bassiana* 1 or 2 days prior given no choice.

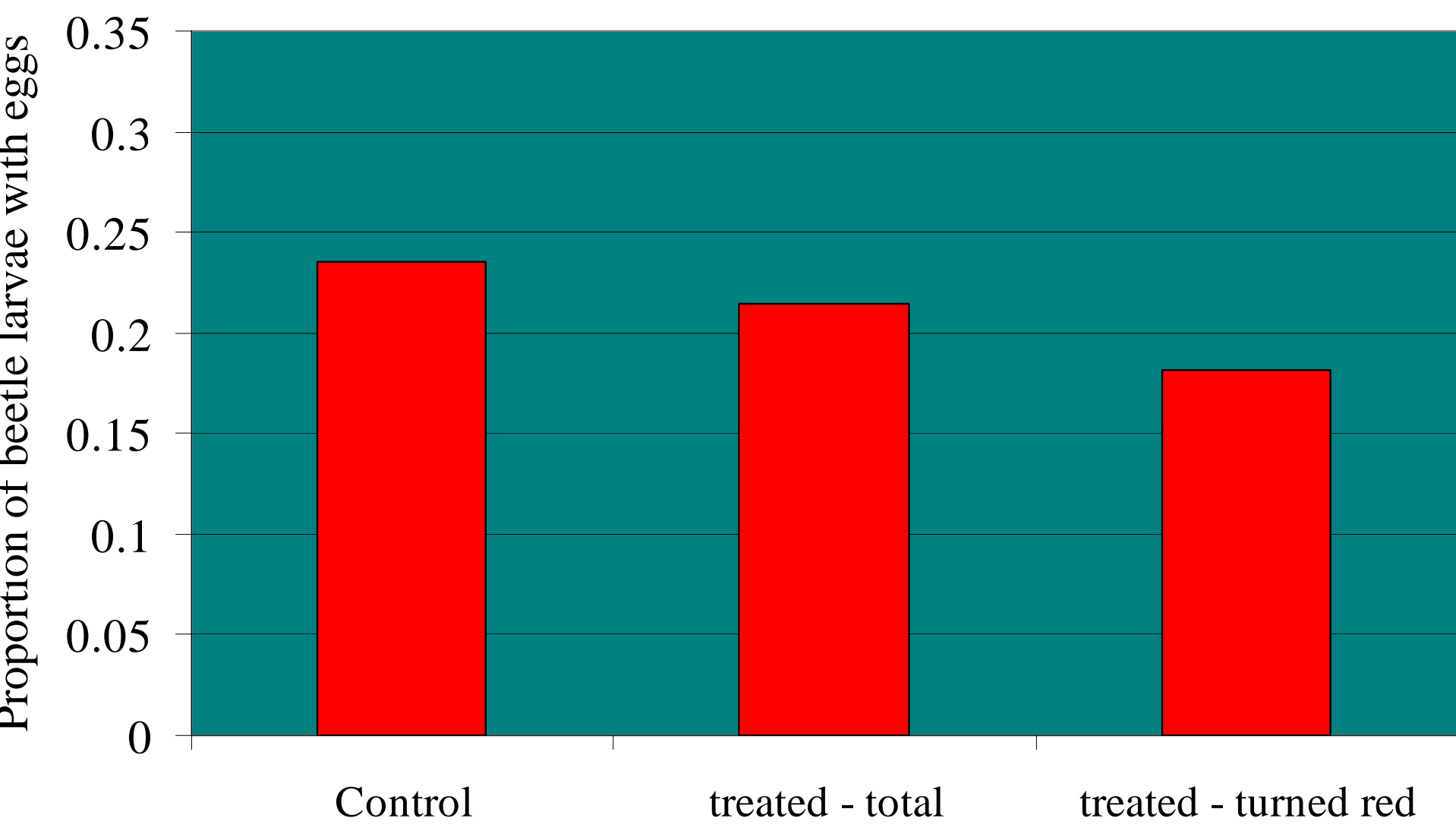


Figure 3. Oviposition choice of *Cephalonomia tarsalis* on *Beauveria bassiana*-treated and untreated sawtoothed grain beetle larvae. Treated larvae were exposed to fungus for 24-48 hours. Those that turned red from fungus metabolites did so during the 24 hour oviposition period.

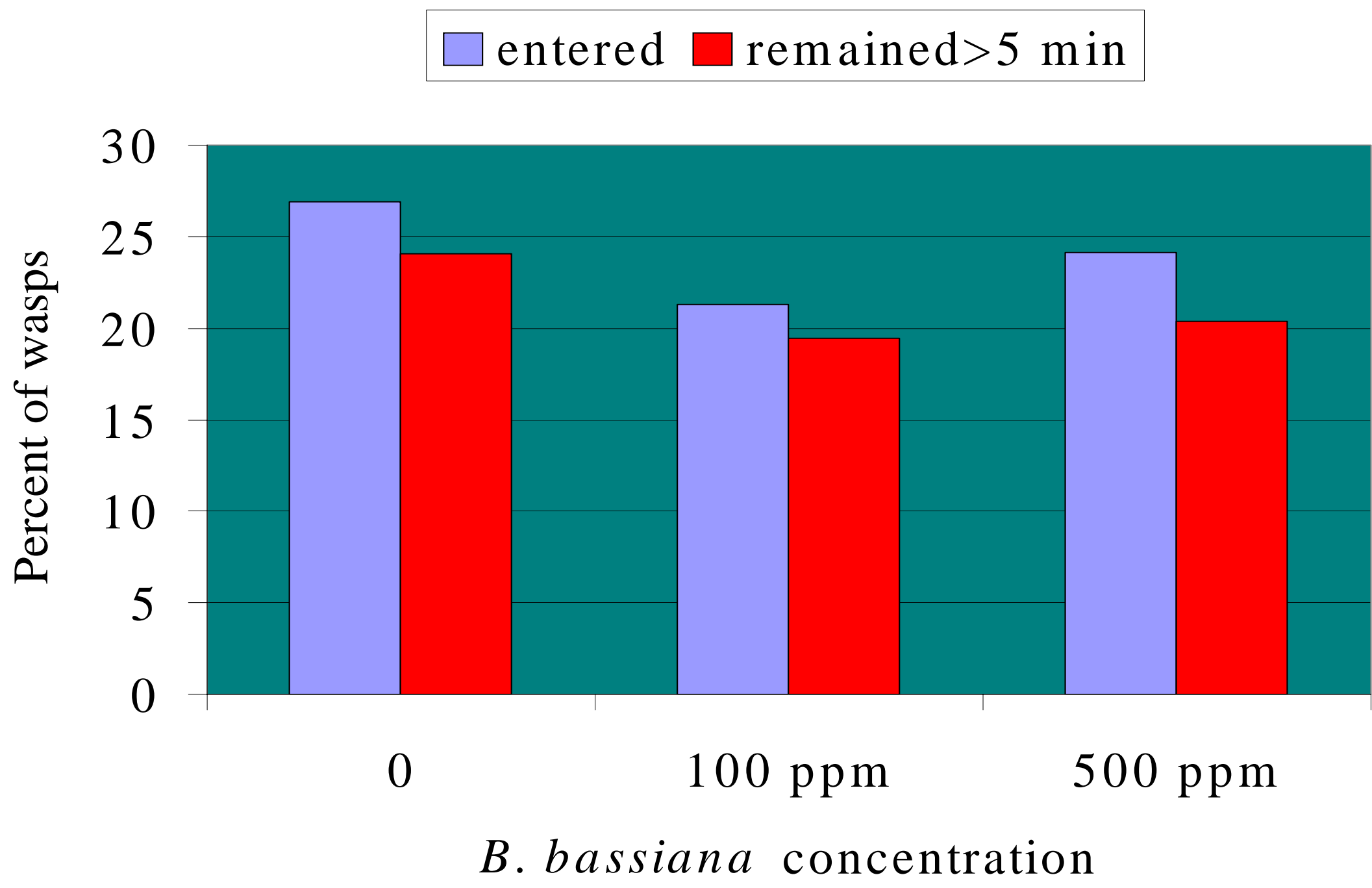


Figure 4. Entry of *C. tarsalis* females into *Beauveria bassiana*-treated wheat

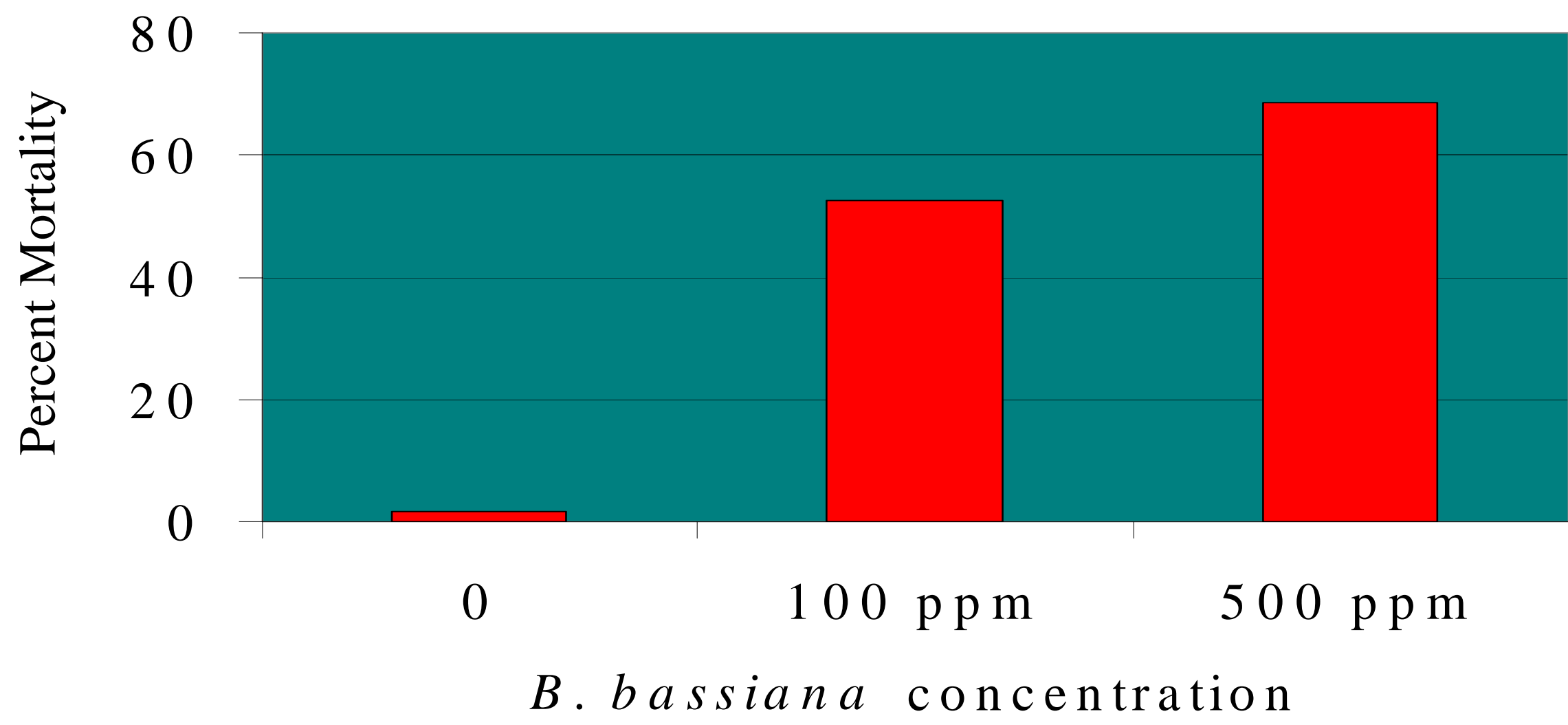


Figure 5. Mortality of *Cephalonomia tarsalis* females exposed to *Beauveria bassiana* conidia for 3 hours and incubated for 8 days at 26°C and 75% RH.

Results and Discussion

Female wasps oviposited on *B. bassiana*-infected sawtoothed grain beetle larvae until within a day of host death and the appearance of red pigment, oosporein (Figs. 1 and 2). Wasp progeny deposited on fungus-infected hosts all died within 2 days of oviposition (Fig. 1). The small decrease in oviposition on beetles with mycosis, compared to controls, can be attributed to loss of host suitability and probably does not reflect detection of the specific disease. When the wasps were given a choice between *B. bassiana*-treated and untreated hosts, the percent of individuals that oviposited was 23.5 (SD 9.2) for control hosts, 21.5 (SD 5.9) for treated hosts that did not show visible signs of mycosis, and 18.1 (SD 3.2) for hosts that had red pigment within the 24 hour oviposition period (Fig. 3).

Female wasps entered wheat treated with fungus spores with approximately the same frequency that they did into clean wheat even at a concentration of 500 ppm and visually detectable spore dust (Fig. 4). Three-hour exposures to the 100 ppm and 500 ppm test concentrations resulted in 52.5 and 68.6% mortality, respectively, contrasting with only 1.7 % mortality among control wasps (Fig 5).

Clearly, *C. tarsalis* adult females are susceptible to *B. bassiana* on a level that is comparable to the susceptibility of the their hosts. Also, wasps eggs that are deposited on hosts with mycosis do not survive.

Although both larval and adult *C. tarsalis* are susceptible to *Beauveria bassiana*, the wasps are clearly not able to detect and avoid the fungus. In at least two parasitoid-host-fungus systems, the wasps have evolved the ability to discriminate between suitable hosts and those with mycosis (Fransen and van Lenteren 1993 Entomol. Exp. Appl. 69:239-49; Jones and Poprawski 1996, IOBC/OILB Int. Conf. p. 216). Those associations involve organisms at are likely to encounter one another in nature. The natural concentrations of *B. bassiana* in stored grain are not likely to be high enough to cause death. The inability of *C. tarsalis* to avoid the fungus may simply reflect lack of selection pressure.